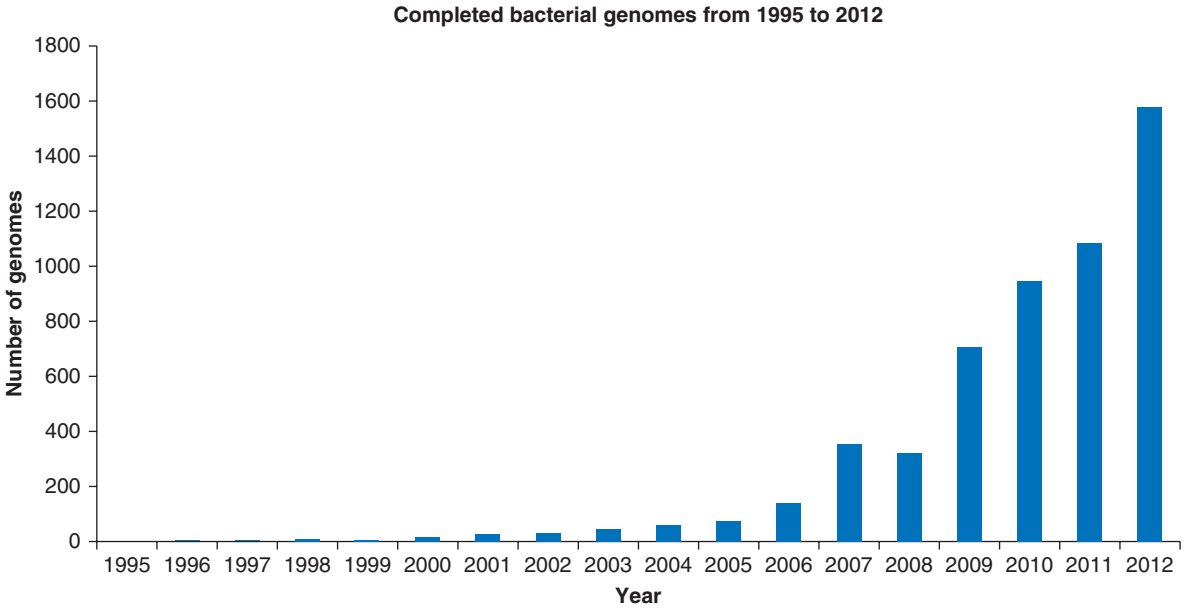

FIGURE 146-1 Completed bacterial genome sequences by year, through 2012. *(Data compiled from www.genomesonline.org.)*

acid–based diagnostics promise improved speed, sensitivity, specificity, and breadth of information. Bridging clinical and research laboratories, adaptations of genomic technologies have begun to deliver on this promise.

HISTORICAL LIMITATIONS AND PROGRESS THROUGH GENETIC APPROACHES

The molecular diagnostics revolution in the clinical microbiology laboratory is well under way, borne of necessity in the effort to identify microbes that are refractory to traditional culture methods. Historically, diagnosis of many so-called unculturable pathogens has relied largely on serology and antigen detection. However, these methods provide only limited clinical information because of their suboptimal sensitivity and specificity as well as the long delays that diminish their utility for real-time patient management. Newer tests to detect pathogens based on nucleic acid content have already offered improvements in the select cases to which they have been applied thus far.

Unlike direct pathogen detection, serologic diagnosis—measurement of the host's response to pathogen exposure—can typically be made only in retrospect, requiring both acute- and convalescent-phase sera. For chronic infections, distinguishing active from latent infection or identifying repeat exposure by serology alone can be difficult or impossible, depending on the syndrome. In addition, the sensitivity of serologic diagnosis varies with the organism and the patient's immune status. For instance, tuberculosis is notoriously difficult to identify by serologic methods; tuberculin skin testing using purified protein derivative (PPD) is especially insensitive in active disease and may be cross-reactive with vaccines or other mycobacteria. Even the newer interferon γ release assays (IGRAs), which measure cytokine release from T lymphocytes in response to *Mycobacterium tuberculosis*–specific antigens in vitro, have limited sensitivity in immunodeficient hosts. Neither PPD testing nor IGRAs can distinguish latent from active infection. Serologic Lyme disease diagnostics suffer similar limitations: in patients from endemic regions, the presence of IgG antibodies to *Borrelia burgdorferi* may reflect prior exposure rather than active disease, while IgM antibodies are imperfectly sensitive and specific (50% and 80%, respectively, in early disease). The complex nature of these tests, particularly in view of the nonspecific symptoms that may accompany Lyme disease, has had substantial implications on public perceptions of Lyme disease and antibiotic misuse in endemic areas. Similarly, syphilis, a chronic infection caused by *Treponema pallidum*, is notoriously difficult to stage by serology alone, requiring the use of multiple different nontreponemal (e.g., rapid protein reagin) and treponemal (e.g., fluorescent treponemal antibody) tests in conjunction with clinical suspicion. Complementing serology, antigen detection can improve sensitivity and specificity in select cases but has been validated only for a limited set of infections. Typically, structural elements of pathogens are detected, including components of viral envelopes (e.g., hepatitis B surface antigen, HIV p24 antigen), cell surface markers in certain bacteria (e.g., *Streptococcus pneumoniae*, *Legionella pneumophila* serotype 1) or fungi (e.g., *Cryptococcus*, *Histoplasma*), and less specific fungal cell-wall components such as galactomannan and β-glucan (e.g., *Aspergillus* and other dimorphic fungi).

Given the impracticality of culture and the lack of sensitivity or sufficient clinical information afforded by serologic and antigenic methods, the push toward nucleic acid–based diagnostics originated in pursuit of viruses and fastidious bacteria, becoming part of the standard of care for select organisms in U.S. hospitals. Such tests, including polymerase chain reaction (PCR) and other nucleic acid amplification tests (NAATs), are now widely used for many viral infections, both chronic (e.g., HIV infection) and acute (e.g., influenza). This technique provides essential information about both the initial diagnosis and the response to therapy and in some cases genotypically predicts drug resistance. Indeed, progression from antigen detection to PCR transformed our understanding of the natural course of HIV infection, with profound implications for treatment (Fig. 146-2). In the early years of the AIDS pandemic, p24 antigenemia was detected in acute HIV infection but then disappeared for years before emerging again with progression to AIDS (Fig. 146-2*B*). Without a marker demonstrating viremia, the role of treatment during HIV infection prior to the development of clinical AIDS was uncertain, and monitoring treatment efficacy was challenging. With the emergence of PCR as a progressively more sensitive test (now able to detect as few as 20 copies of virus per milliliter of blood), viremia was recognized as a near-universal feature of HIV infection. This recognition has been transformative in guiding the initiation of therapy as well as adjustments in therapy and, together with the development of less toxic therapies, has helped to shape guidelines that now favor earlier introduction of antiretroviral therapy for HIV infection.

As they are for viruses, nucleic acid–based tests have become the diagnostic tests of choice for fastidious bacteria, including the common sexually transmitted intracellular bacterial pathogens *Neisseria gonorrhoeae* and *Chlamydia trachomatis* as well as the tick-borne *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*. More recently, nucleic acid amplification–based detection has offered improved sensitivity for diagnosis of the important nosocomial pathogen *Clostridium difficile*; NAATs can provide clinically relevant information on the presence of cytotoxins A and B as well as molecular markers of hypervirulence such as those characterizing the recently recognized North American pulsotype 1 (NAP1), which is found more frequently in